STUDIES ON THE QUALITY OF THE PLANT FOOD II. INFLUENCE OF VARIETY AND ENVIRONMENT UPON THE RIBOFLAVIN CONTENT OF HULLED RICE (2) IN SPECIAL REFERENCE TO ITS EFFECTIVE FACTORS

著者	SONE Kiyohide
journal or publication title	Tohoku journal of agricultural research
volume	9
number	3
page range	179-197
year	1959-01-30
URL	http://hdl.handle.net/10097/29251

STUDIES ON THE QUALITY OF THE PLANT FOOD II. INFLUENCE OF VARIETY AND ENVIRONMENT UPON THE RIBOFLAVIN CONTENT OF HULLED RICE (2) IN SPECIAL REFERENCE TO ITS EFFECTIVE FACTORS

By

Kiyohide SONE

Department of Living Science, Faculy of Agriculture, Tohoku University, Sendai, Japan

(Received May 24, 1958)

I. Introduction

In the case of plant foods, it has been shown by Crane (1), Mangelsdorf (2), LeRosen (3), Walker (4) and Sugawara (5) that the content of ascorbic acid and provitamin A varies with the difference of genes and the organization of genomes, the polyploydy of chromosomes and the difference of sex organs of spinach. On the other hand, it has been pointed out by Wade (6), Gastler (7), Krober (8) and Tandon (9) that the vitamin contents in plant foods are considerably influenced by the difference of growth conditions of the plants. These workers (6~9) investigated the nutritive values of plant foods in connection with the varieties of the plants and three other factors of culture after they had divided the condition of the environment into three factors: the fertility and the geographical location of the experimental field and the year of planting. But, considering from the report of Adams (10) on the contamination of the air by unfavorable sorts of gas, it is easily inferred that the vitamin production of plants is also influenced by injurious components which are contained in the gas. Peterson (11) and his coworkers reported that the quantity of carotene and riboflavin increases in the leaves of soybean under the existence of phosphorus. If this is true, it may be inferred in the case of rice plants that the difference of riboflavin contents in the kernels depends on the difference of fertilizers from the reports of Tanitazawa (12) and Sone (13). Part of the amount of insecticide components and other chemicals of agriculture, which are sprayed or dusted on the plants to protect them from biological deseases and damages, is rapidly absorbed through the stems and leaves, and then accumulated in hipocotyls, epicotyls and terminal buds (14), but their residue is absorbed in the soil colloid (15) and the greater part of them is distributed by the plow and cultivation depth of soil (16). Then the effective components of the organic insecticide distributed in the plow and cultivation depth of soil, vanish by evaporation and decomposition by soil microorganisms (15), but up to this time a greater amount of the components of insecticides and other chemicals are absorbed through the roots and leaves, some of which turn to be complex in leaves. It is, therefore, possible that the flavor and taste of plant foods are influenced by the treatment of insecticides and other chemicals as reported by Dawson (17), Reynolds (18), Kirkpatrick (19), Gilpin (20) and Birdsall (21). Considering the fact that the riboflavin biosynthesis of microorganisms is influenced by the existence of heavy metallic ions as Fe^{++} and Pb^{++} [see Katagiri (22) and Rokusyo (23)], it is probable that the elements of halogen and heavy metals contained in the insecticides and other chemicals of agriculture influence the biosynthesis of vitamins in plants. Considering these facts, the effective factors which influence the vitamin content in plant foods are given as follows:

Natural Factors
1) Variety of the plant (Hereditary)
2) Location of the experimental field (Geographical)
3) Weather of the experimental station
4) Year of planting
5) Kind of soil

Artificial Factors
1) Fertilizer
2) medicine (e.g.; Insecticide, Fungicide, Herebicide)
3) Other Chemicals

Among these factors, the year is closely related with the weathar, and the kind of soil is not distinguished from the locality sometimes, and the influence of medicine is apt to be forgotten in general. Even though the same material is applied at the time of the above mentioned experiment it is not uncommon to give different results. In this paper, the effective factors which influence the riboflavin content in plant foods are agronomically investigated by using the rice plant which is closely related to our food.

II. Materials and Methods

The total number of the varieties of rice plants used in this experiment was fifteen and they were raised in the fields of five experimental stations at four locations in Miyagi Prefecture according to the purpose of each experiment. In the investigation of varietal and environmental differences of riboflavin contents in the various kinds of rice, factors excluding the effective ones for the object were controlled as carefully as possible. The details of experiment materials and methods of their management will be described in each item.

III. Influences of Natural Factors

(1) *Materials*

Thirteen varieties of rice plants shown in Table 1 were raised in the paddy fields of three locations in Miyagi Prefecture.

Table 1. Experimental stations and varieties of rice-plants.

Experimental station		Furukawa	Sendai		Iwanuma	
Variety \ Soil		S	S	S	S	P
Variety \ Year		1955	1954	1955	1955	1955
E	Norin No. 16	+	+	+		
	Fujisaka No. 5	+	+	+	+	+
	Rikuu No. 132	+	+	+		
	Ayashi No. 2	+		+		
M	Norin No. 17		+	+	+	+
	Norin No. 24	+	+	+	+	+
	Ayashi No. 1	+	+	+		
	Tohoku No. 14	+	+	+	+	+
	Yachikogane			+	+	+
L	Norin No. 49	+		+		
	Norin No. 50		+	+	+	+
	Shin No. 2	+		+	+	
	Aikoku No. 1	+			+	+

Furukawa : Furukawa Branch of Miyagi Agricultural Experiment Station (Northern part of Miyagi Prefecture)
Sendai : Miyagi Agricultural College (Central part of Miyagi Prefecture)
Iwanuma : Iwanuma Branch of Miyagi Agricultural Experiment Station (Southern part of Miyagi Prefecture)
E : Early variety, M : Inter Mediate variety, L : Late variety. S : Clayey loam, P : Peat soil.

Fertilizers were applied as shown in Table 2 considering the conditions of geographical location and the weather of each experiment Station.

Table 2. Conditions of fertilizers.

Fertilizer	Nursery-bed (Monme/Tsubo)			Paddy-Field (Kan/Tan)		
	Furukawa	Sendai[1]	Iwanuma	Furukawa	Sendai[2]	Iwanuma[3]
N	16	12	13	1.400	1.800	1.640
P_2O_5	12	11	10	1.000	1.700	1.370
K_2O	12	12	8	0.720	1.700	0.940
Kind[4]	A C E F H	A C E	A C E H	A C F H	A B D E H	A C E G H

1) Same conditions of the fertilizers in 1954 and 1955.
2) Same conditions of the fertilizers in 1954 and 1955.
3) Same conditions of the fertilizers in P and S.
4) A : Ammonium sulfate B : Calcium cyanamide C : Calcium superphosphate
D : Fused phosphate E : Potassium chloride F : Potassium sulfate
G : Lime H : Farmyard-manue

1 Monme = 3.7500 g, 1 Kan = 3.7500 kg = 8.2672 lb. 1 Tan = 300 Tsubo = 154.2 a = 0.2451 acre

Table 3. Time of planting, growth and others,

Experimental station	Furukawa				Sendai			
Soil	S				S			
Year	1955				1954			
Date of seeding	April, 15				April, 23			
Date of transplanting	June, 1				June, 8			
Variety	Date			Weight of 1,000 kernels	Date			Weight of 1,000 kernels
	Heading	Ripening	Harvest		Heading	Ripening	Harvest	
Norin No. 16	Aug. 4	Sept. 17	Sept. 20	18.3g	Aug. 23	Sept. 28	Oct. 15	16.9g
Fujisaka No. 5	July. 31	" 7	" 16	20.0	" 17	" 20	" 15	18.5
Rikuu No. 132	Aug. 5	" 19	" 22	19.4	" 24	" 29	" 15	18.7
Ayashi No. 2	" 1	" 10	" 16	18.7	—	—	—	—
Norin No. 17	—	—	—	—	" 23	" 28	" 15	18.8
Norin No. 24	" 9	" 26	" 27	19.8	" 27	Oct. 1	" 15	19.9
Ayashi No. 1	" 4	" 24	" 27	20.2	" 24	Sept. 30	" 15	19.5
Tohoku No. 14	" 4	" 24	" 27	18.8	" 24	" 29	" 15	19.0
Yachikogane	—	—	—	—	—	—	—	—
Norin No. 49	" 8	" 23	" 27	20.5	—	—	—	—
Norin No. 50	—	—	—	—	" 29	Oct. 5	" 15	19.5
Shin No. 2	" 15	" 25	" 27	19.2	—	—	—	—
Aikoku No. 1	" 15	" 30	Oct. 3	19.8	—	—	—	—

* Weight of dry 1,000 kernels of hulled rice.

The conditions of planting and culture of the sample rice plant, the time of growth and others and the weight of 1,000 kernels of each varieties are shown in Table 3.

Rice plants which were cultured and harvested according to the above conditions shown in Tables 1~3 were airdried sufficiently in the field of each experiment station. Then, they were threshed and husked. Completely hulled grains of rice were bolted out to make uniform carac districts of the kernels, so that the breadth of each kernel becomes within the range of 2.0 mm to 2.2 mm, according to the report of Okamura (24), stored in a colored desiccator at a cold and dark place, and then applied to the following experiment.

(2) *Estimation of Moisture and Total-riboflavin in Hulled Rice*

Moisture: The moisture was estimated at 100~105°C by the customary method.
Total-riboflavin: The total-riboflavin was estimated by the fluorometric assay of lumiflavin, which was described in the previous report (13).

(3) *Results and Discussion*

Variety and Locality

Rerults of the actual estimation of the total riboflavin content in the hulled rice obtained at the three experiment stations, Furukawa, Sendai and Iwanuma, are shown in Table 4.

The riboflavin amounts in the different varieties of hulled rice harvested weight of 1,000 kernels of hulled rice.*

Sendai S 1955 April, 20 June, 8				Iwanuma S 1955 April, 20 June, 1				Iwanuma P 1955 April, 20 June, 1			
Date			Weight of 1,000 kernels	Date			Weight of 1,000 kernels	Date			Weight of 1,000 kernels
Heading	Ripening	Harvest		Heading	Ripening	Harvest		Heading	Ripening	Harvest	
Aug. 3	Sept. 20	Oct. 15	17.8g	—	—	—	—g	—	—	—	—g
〃 2	〃 15	〃 15	19.5	July 26	Sept. 7	Sept. 13	20.1	July 29	Sept. 8	Sept. 13	20.1
〃 5	〃 15	〃 15	19.4	—	—	—	—	—	—	—	—
〃 2	〃 15	〃 15	19.0	—	—	—	—	—	—	—	—
〃 4	〃 20	〃 15	19.6	Aug. 3	〃 14	〃 18	20.2	Aug. 5	〃 16	〃 18	20.0
〃 8	〃 23	〃 15	20.2	〃 6	〃 18	〃 18	20.5	〃 7	〃 20	〃 18	20.6
〃 4	〃 19	〃 15	20.5	—	—	—	—	—	—	—	—
〃 4	〃 19	〃 15	19.2	〃 1	〃 11	〃 13	18.3	〃 3	〃 12	〃 13	19.3
July 31	〃 15	〃 15	18.4	〃 21	Oct. 4	Oct. 7	19.3	〃 24	Oct. 1	Oct. 7	19.1
Aug. 5	〃 20	〃 15	19.6	—	—	—	—	—	—	—	—
〃 15	〃 30	〃 15	20.3	〃 13	Sept. 22	Sept. 25	18.2	〃 12	Sept. 24	Sept. 25	20.7
〃 9	〃 28	〃 15	19.6	〃 7	〃 19	〃 25	19.5	—	—	—	—
—	—	—	—	〃 14	〃 26	〃 25	19.7	〃 1	〃 27	〃 25	19.8

Table 4. Content of total-riboflavin in hulled rice. (I)
(μg per 100 g of dry hulled rice)

Experimental station		Furukawa	Sendai	Iwanuma	Average of Each Variety
Variety \ Soil		S	S	S	
E	Norin No. 16	52	50	—	51
	Fujisaka No. 5	56	47	55	53
	Rikuu No. 132	48	49	—	49
	Ayashi No. 2	53	47	—	50
	Average	52	48	55	51
M	Norin No. 17	—	57	51	54
	Norin No. 24	50	65	54	56
	Ayashi No. 1	52	59	—	56
	Tohoku No. 14	46	59	50	52
	Yachikogane	—	53	55	54
	Average	49	59	53	54
L	Norin No. 49	60	66	—	63
	Norin No. 50	—	54	48	51
	Shin No. 2	48	77	48	58
	Aikoku No. 1	63	—	52	58
	Average	57	66	49	58
Average of Each Station		53	57	52	

at three experiment stations are compared thus;

at Furukawa Experiment Station L>E>M

at Sendai Experiment Station L>M>E

at Iwanuma Experiment Station M>L

Although the tendency recognized in the above comparison differs in each station, the general tendency, roughly speaking, may be said to be:

L > M > E

From the above results, it is conceivable that the increase of the riboflavin amount differs with the growth of the rice plant. If this is true, this assumption proves the theory of Ferri (25) and Galston (26) that the riboflavin diminishes the influence of indole acetic acid under the existence of light by the fact that it represses the growth of rice plants.

As to the difference of riboflavin contents in hulled rice grown at various locations, the average of riboflavin contents in the Furukawa Experiment Station and that of Iwanuma coincides with each other within the range of experiment error, but, the average of the Sendai Experiment Station is clearly higher than those of the others. No difference of riboflavin content in hulled rice caused by the latitudinal difference is likely to be found within such degree of difference of geographical locations as these. So it is doubtful whether the conclusions of Gastler (7) and Wade (6) — that the difference of geographical location influences remarkably the riboflavin content of barley and cow-pea — is applicable to the case of the rice plant. In other words, it seems more proper to infer that the difference in question is caused not so much by the locational difference — that is, mainly the geographical difference of latitude — as by the various environmental conditions of growth including the factors, such as the kind of sail of an experiment field, of fertilizer, etc.

Crossing of the varieties

The results of the comparsion of the riboflavin contents in varieties of hulled rice with each other from the standpoint of crossing of the varieties are given in Table 5.

Table 5. Content of total-riboflavin in hulled rice. (II)
(μg per 100g of dry hulled rice)

Variety		Norin No. 16	Norin No. 24	Ayashi No. 1	Rikuu No. 132
P	♀ ♂	Banshirozasa Rikuu No. 132	Bantokohai No. 33 Rikuu No. 132	Sanki Rikuu No. 132	Rikuu No. 20 Kamenoo No. 4
Experi-mental station	Furukawa	52	50	52	48
	Sendai	50	65	59	49

The paternal variety to the three varieties, Norin No. 16, Norin No. 24 and Ayashi No. 1, is Rikuu No. 132, but, in the case of Sendai Station, considerable differences of riboflavin contents are found in the four varieties of rice plants, and it is inferred that the paternity of the crossing does not directly affect the riboflavin content in hulled rice in the case the paternal varieties, are common. Of course, more investigations are required about the crossing to conclude on the influence of crossing upon the riboflavin contents of different varieties, but in general, it is conjectured that the varietal differences of riboflavin contents of soybean (27) and hulled rice result from the difference of adaptation to the environment of plant growth.

Soil

The riboflavin content in the hulled rice, which was raised in the fields of different kinds of soil, and under the same conditions of fertilizers, at Iwanuma Experiment Station, is given in Table 6.

Table 6. Content of total-riboflavin in hulled rice. (III)
(μg per 100g of dry hulled rice)

Variety \ Soil	S	P
Fujisaka No. 5	55	53
Norin No. 17	51	73
Norin No. 24	54	61
Tohoku No. 14	50	62
Yachikogane	55	71
Norin No. 50	48	71
Aikoku No. 1	52	88
Average of each kind of soil	52	68

From Table 6, it is found that six varieties of rice plants except Fujisaka No. 5 give similarly higher values of the riboflavin contents in the field of peat soil than in the field of clayey loam. In general, the average value of riboflavin content in hulled rice is higher when the rice plants are raised in the field of peat soil. The maximum amount of the varietal differences of riboflavin contents in hulled rice raised in the field of the same kind of soil is 7 μg in the field of clayey loam, but 35 μg in the field of peat soil, and thus the influence of the soil is clearly found. The minimum value of the differences of riboflavin contents in the same variety raised in the fields of the different kinds of soil is 2 μg (Fujisaka No. 5) and the maximum is 36 μg (Aikoku No. 1), and thus it is conjectured that the special character of the variety is considered to appear more remarkably when it is raised in the field of peat soil than in the field of clayey loam.

Year

The riboflavin contents in the hulled rice raised under the condition of the same varieties and the same fertilizers in the same paddy field in Sendai Station in 1954 and 1955, are shown in Table 7.

Table 7. Content of total-riboflavin in hulled rice. (IV)
(μg per 100g of dry hulled rice)

Variety \ Year		1954	1955
Norin	No. 16	48	50
Fujisaka	No. 5	41	47
Rikuu	No. 132	47	49
Norin	No. 17	44	57
Norin	No. 24	45	65
Ayashi	No. 1	47	59
Tohoku	No. 14	52	59
Norin	No. 50	44	54
Average of each year		46	55

As to the riboflavin contents in Norin No. 16 and Rikuu No. 132 (both hulled), the influence of the year of growth is not recognized, but, in the other varieties the riboflavin contents are similarly higher in 1955 than in 1954, and the influence of the year of growth is found in the riboflavin content in the hulled rice of paddy rice plants. In this case, the differences of the riboflavin contents differentiated by the years of the growth are to be considerd to result from the difference of the weather, because the conditions of the locality, fertilizer and field (i.e., mainly the kind of soil) are similar.

Though the riboflavin content of each variety of hulld rice is not the same because the adaptability of each differs to the environment of the growth of rice plants, it is considered to be adequate to assume that the varietal difference influences comparatively stronger the riboflavin content in hulled rice in the field of clayey loam than the differences of the locality and year. Furthermore, the special characters of the varieties appear more remarkable in the case of the paddy field of peat soil.

III. Influences of Artificial Factors

1. Fertilizers

(1) *Materials*

The conditions of cultivation of paddy rice plants are shown in Table 8, considering the conditions of the locality and weather. The experiment was done on about three elements of fertilizers, i.e. nitrogen, phosphorus and potassium, and five plots, of non-fertilizer, non-nitrogen, non-phosphorus, non-potassium and perfect-fertilizers, were settled.

The seedling of the sample rice plants, which were seeded in the same nursery bed and cultured under the same conditions, were transplanted at the same time in each plot in each experiment station. The date of seeding, transplanting and havest, are shown in Table 9.

The changes of pH value of the soil during the experiment at Iwanuma

Station are shown in Table 10.

Rice plant were cultured and harvested in accordance with the conditions of Tables 8~10, and then airdried satisfactorily in the field of each experiment station. And after threshing, and husking, only perfect hulled grains of rice

Table 8. Conditions of cultivation.

Experimental station		Soil	Variety	Fertilizer		
				Amount (Kan/Tan)		Kind[4]
Sendai I[1]		Clayey loam	Narin No. 24	N P_2O_5 K_2O	1.064 1.552 0.385	A C E
Sendai II[2]		Muck soil	Norin No. 21	N P_2O_5 K_2O Farmyard-manue	2.000 2.000 2.000 100.000	B D E
Iwanuma[3]	I	Clayey loam	Norin No. 24	N P_2O_5 K_2O	1.600 2.500 1.500	B D E
	II	Peat soil				

1) Sendai I : Kita-Rokubanchyo, Sendai City, Miyagi Prefecture.
2) Sendai II : Shichigo, Sendai City, Miyagi Prefecture.
3) Iwanuma : Natori Town, Miyagi Prefecture.
4) A : Ammonium sulfate C : Calcium superphosphate E : Potassium chloride
B : Urea D : Fused phosphate

Table 9. Date of seeding, transplanting and harvest.

Experimental station		Date		
		Seeding	Transplanting	Harvest
Sendai	I	Apr., 20	June, 5	Oct., 7
Sendai	II	〃 18	〃 5	Sept., 26
Iwanuma	I	〃 26	〃 8	Oct., 4
Iwanuma	II	〃 26	〃 8	〃 3

Note: As to the date of heading in each plot, no notable differences were found in each experimental station.

Table 10. Change of pH value of the soil.

Soil	Plot	pH		
		June, 25	Aug., 11	Oct., 24
Clayey loam	None	6.05	6.16	6.05
	P K	6.27	6.84	6.48
	N K	5.95	6.68	6.02
	N P	6.36	6.93	6.14
	N P K	6.16	6.73	6.40
Peat soil	None	5.68	6.28	6.16
	P K	5.94	6.30	6.15
	N K	5.85	6.22	6.21
	N P	5.87	6.37	6.23
	N P K	6.07	6.38	6.27

were gathered and stored in a colored desiccator in a cold and dark place, then they were applied to the following estimation.

(2) *Method*

As to the estimation of moisture and total riboflavin content, the same method, as was applied in the above mentioned experiment, was undertaken in this experiment.

(3) *Results and Discussion*

Yield

The weight of 1,000 kernels, that of 1 Sho, the yield per 1 Tan, and the ratio of waste rice, of the hulled rice ripened under the conditions of cultivation described above are shown in Table 11.

Table 11. Results of the survey on the yield of hulled rice.

Experimental station	Plot	Weight* of 1,000 kernels (g)	Weight* of 1 Syo (kg)	Yield* per 1 Tan (kg)	Ratio of waste rice (%)
Sendai I	None	19.5	—	—	—
	P K	19.3	—	—	—
	N K	20.0	—	—	—
	N P	19.7	—	—	—
	N P K	19.9	—	—	—
Sendai II	None	20.0	1.21	272	—
	P K	19.9	1.19	282	—
	N K	20.1	1.20	267	—
	N P	19.9	1.20	335	—
	N P K	20.1	1.23	364	—
Iwanuma I	None	20.4	1.27	200	2.3
	P K	20.2	1.24	231	1.5
	N K	20.4	1.24	294	4.3
	N P	20.5	1.22	293	3.7
	N P K	20.9	1.27	310	3.1
Iwanuma II	None	20.7	1.22	276	2.2
	P K	20.6	1.21	286	1.7
	N K	20.4	1.20	330	2.3
	N P	20.5	1.20	283	3.0
	N P K	20.6	1.20	318	2.9

* Weight of dry matter of hulled rice.

a) Weight of 1,000 kernels of hulled rice

In the Iwanuma Station, rice kernels both of the Field I and the Field II seemed to be a little more (comparatively) ripened than in the Sendai Station, but, the difference of each to be seen among the weights of kernels in each plot was not so remarkable in each station.

b) Weight of 1 Sho

Weight of 1 Sho in the Field I in the Iwanuma Station seemed to be a little higher than that in the Field II in the Sendai Station and the Field II in the Iwanuma Station, but the difference of this degree is found even in the same plot, and so it has no significance. Also no tendency in the difference of weight to be found among the plots is notable, in each experiment station.

c) Yield per 1 Tan

Results of the comparison of the yields of hulled rice per 1 Tan in all the plots in each station are considered as follows:

in the Field II of Sendai NPK>NP>PK, None, NK, and

in the Field I of Iwanuma NPK>NP, NK>PK>None,

and the influence of fertilizers appears comparatively significant in this case, but, in the Field II of Iwanuma, it is possible to consider the order of the yield as follows:

NK>NPK>NP, PK>None.

Thus, it seems that the influence of fertilizers partially differs from the results obtained in the Field II of Sendai and the Field I of Iwanuma. Furthermore, it is naturally expected that the riboflavin content of None gives the low value in each field in this experiment, but, it is not yet certain whether the fact that the content of riboflavin in hulled rice gives the lower value in the plot of NPK than in the plot of NK in the Field II of Iwanuma results from the special character of the peat soil.

d) Ratio of waste rice

The ratio of waste rice in each plot of each station seemes to be in the following order:

in the Field I of Iwanuma NPK>PN, NK>PK>None. and

in the Field II of Iwanuma NPK, PN>NK, None>PK,

and it is found that the ratio in the plots of PK and None occur in both cases of clayey loam and peat soil. Therefore, it is conjectured that uncertainty of the tendency of the wast rice ratio in the plots of NPK, NK and PN according to the kind of the soil results from the difference of the effect of nitrogen to the soil.

Relation between the Amount of Riboflavin in Hulled Rice and the Fertilizers

The amount of riboflavin in hulled rice is shown in Table 12.

a) The amount of riboflavin per 1,000 kernels and 100 g of hulled rice.

The amount of riboflavin per 1,000 kernels and 100 g of hulled rice seems to be in the following order:

in the Field I of Sendai NK, NP, None, NPK>PK

in the Field II of Sendai NPK>PK, NK, NP>None

in the Field I of Iwanuma NK>PK, NP>None, NPK

in the Field II of Iwanuma NP, PK>NPK>None>NK,

and it is seemingly true that the phosphorus particularly affects the increase of riboflavin in hulled rice as previously reported by Peterson (11) in the case

Table 12. Content of riboflavin in hulled rice and amount of riboflavin production. (V)

Experimental station	Plot	Total-riboflavin		
		μg/1,000 kernels	μg % *	mg/Tan
Sendai I	None	11.1	56.8	—
	P K	10.0	52.1	—
	N K	11.4	56.9	—
	N P	11.3	57.3	—
	N P K	11.1	55.8	—
Sendai II	None	9.0	45.1	122
	P K	10.5	52.6	149
	N K	10.1	50.3	133
	N P	10.3	52.0	174
	N P K	12.2	60.6	202
Iwanuma I	None	10.2	50.0	110
	P K	11.6	57.5	133
	N K	12.2	59.9	176
	N P	11.6	56.8	166
	N P K	10.5	50.3	156
Iwanuma II	None	11.2	54.3	150
	P K	12.1	58.9	168
	N K	10.7	52.5	173
	N P	12.4	60.5	171
	N P K	11.7	56.8	180

* μg per 100g of dry hulled rice

of muck soil and peat soil, but, no distinct tendency is found in the other plots.

b) The amount of the riboflavin production per one Tan

The amount of riboflavin obtained from the field of one Tan, seems to be in the following order:

in the Field II of Sendai NPK>NP>PK>NK>None,
in the Field I of Iwanuma NK>NP>NPK>PK>None,
in the Field II of Iwanuma NPK>NK, NP, PK>None.

And the relation of the plot of NPK and that of NK is opposite to the relation which is supposed from the result of the investigation about the yield of hulled rice per one tan, but, this relation is taken to be a matter of course, considering from the amounts of riboflavin of the 1,000 kernels, and this result is conjectured to have resulted from the specific character of the soil.

Namely, the influence of a fertilizer upon the production of riboflavin is comparatively notable in the case of NK and it is thus conjectured that the phosphorus plays important roles in the production of riboflavin of the paddy rice plant with nitrogen, although the tendency of the production of riboflavin differs according to the kind of soil.

pH and the Soil

Judging from Tables 10 and 12, the changes of pH value in this extent does not exert so remarkable influence upon the riboflavin content in hulled rice, and also the influence of fertilizers upon the changes of pH value is not notable.

Variety and Fertilizer

From Table 4 and Table 12, Table 13 is derived.

Table 13. Content of total-riboflavin in hulled rice. (VI)
(μg per 100g of dry hulled rice)
1955 Iwanuma

Factors	Soil	Maximum	Minimum	Average
Variety*	S P	55 88	48 53	52 68
Fertilizer	S P	60 61	50 53	55 57

* 7 varieties.

No significant difference seems to exist in the cases of variety-clayey loam and of fertilizer-peat soil, but, it is permissible to think that a significant difference exists in the other cases. The difference of the influence that the variety or the fertilizer exerts can hardly be found in the case of clayey loam, but, the superior effectiveness of the variety is clearly found in the case of peat soil. Thus, it is found, as mentioned above, that the content of riboflavin in hulled rice is markedly affected by the difference of rice variety under unfavorable conditions of the environment.

2. Fungicide

(1) *Materials*

Hokuriku No. 14 was raised in the experiment field of clayey loam at Shibata in Miyagi Prefectue and cultured by the customary method. Experiments plots, kinds of fungicide and methods of dusting are shown in Table 14.

Table 14. Experiment plots and fungicides.

Plot	Fungicide	Components	Amount of effective metal	
			Hg	Cu
Cu-Hg I	Cupper mercury dust	Mercury phenyl dust, Basic copper sulfate	0.2%	2.0%
Cu-Hg II	Merdo dust No. 3	Mercury phenyl dust, Yellow copperous oxide	0.2	2.0
Hg I	Tohoku mercury dust	Mercury phenyl dust	0.25	0.0
Hg II	Ceresan dust	Mercury phenyl dust	0.25	0.0
Control	None	—	0.00	0.0

Note: Method of dusting
Fungicides were dusted six times in each plot: July 12, 13; August 2, 13, 23 and September 5. A motive dusting machine was used for this purpose, and 3 kg of the above dusts were applied per one tan every time.

All the above fungicides are generally applied as a dusting powder against the blast of rice plants in our countly, and especially, both Mercury-bordo dust and Merdo-dust No. 3 possesses the action of protection of copper beside the action of direct sterilization of mercury. Time of growth of rice plants in each plot is shown in Table 15.

Table 15. Growth dates of rice plants.

Plot	Date		
	Onset of heading	Heading	Harvest
Cu-Hg I	Aug., 17	Sept., 4	Oct., 17
Cu-Hg II	" 17	" 4	" 17
Hg I	" 17	" 4	" 17
Hg II	" 17	" 4	" 17
Control	" 23	" 4	" 17

In every fungicided plot, the beginning date of heading was six days earlier than in the control plot, but no differences of the date of onset of heading were found, because the temperature was comparatively low in this district after August 10 and the date up to the heading was prolonged. Further, from the result of the survey about the status of damages and diseases of the rice plants caused by the dusting of fungicide, the damage by copper contained in the fungicide was observed in the plots, Cu-Hg I and II, especially at the top of the leaves, but on the whole, it was nearly insignificant.

Outbreaks of the "Blast" were comparatively few and three was no "Rotten-neck" in each plot.

The result of the survey on the yield is shown in Table 16.

Table 16. Yield of rice.

Plot	Weight of whole* unhulled rice (g/Tsubo)	Perfect unhulled rice**/Whole unhulled rice (%)	Perfect hulled rice**/Whole unhulled rice (%)	Weight of 1,000 kernels of perfect hulled rice (g)
Cu-Hg I	256	75.5	62.6	22.8
Cu-Hg II	276	75.0	62.5	22.2
Hg I	278	64.5	51.7	22.2
Hg II	216	61.7	51.3	22.8
Control	243	59.7	49.2	21.8

* Unselected ** Selected

The yield of the perfect unhulled rice and the perfect hulled rice in the plots, Cu-Hg I and II, was superior to that in the other plots, but it is difficult to find the difference of the weight of 1,000 kernels of hulled rice among the products of all the plots after the rice plants were harvested voluntarily five places for each one Tsubo, and each in every plot, they were airdried satisfactorily, and threshed and husked, then the hulled rice was put into glass jars, and stored in a cool and dark place after being put into a colored desiccator.

Grains of perfect hulled rice were selected and these served for the following estimation.

(2) *Methods*

Moisture, crude protein, crude fat, total suger, reducing sugar, non-reducing sugar, ash: These components were estimated according to the customary method. (see "Nogei Kagaku Bunseki Syo" (28)).

Inorganic components were estimated spectrometrically by the method of continuous-arc-radiation using the medium size crystal spectroscope.

After a hole of 2.5 mm (diameter) and 3 mm (depth) was bored into a carbon electrode (manufactured by the Nihon Carbon Co. Ltd., 5.5 mm (diameter)), 10 mg of ashed sample was filled in it, and the same carbon electrode was used as an opposite electrode, the top of which was sharpend to 1 mm (diametar). The distance between the electrodes was kept at 2.5 mm and the intermediate irris to 5 mm, and the width of the slit to 0.005 mm., then, a direct current of 8 A-220 V was electrified so that a continuous arc radiated. A photograph of the spectrum on the process-dry-plate (made by the Fuji Kagaku Co. Ltd.) of spectral analysis was taken through the medium size crystal spectroscope.

Thiamine was estimated by the Diazo method of Sakurai (29).

Total riboflavin was estimated by the method of the previous report (15).

(3) *Results and Discussion*

Result of the estimatin of hulled rice by the above mentioned method is shown in Tables 17 and 18 and 19.

Inorganic Components

When these results are compared with Goto's (30), Ti, Pb, Ag, Mo and Ni are newly found in the hulled rice but Ba is not found in this case, and a trace amount of Sr is found in all plots this time instead of Sr which was found by Goto in the degree below 0.001 per cent of ash in hulled rice. Furthermore, Pb, Sn, Mo and Ag, which are not found in the control plot, and considerable amounts of Zn were found in the fungicided plots when the distribution of the metallic components in hulled rice of each plot is compared with other plots. Hg, which is the main component in the fungicide, was not found by the method of arcradiation which was applied this time, and the difference is not found among the amounts of copper of the plots. But considering from the fact that the Pb, Sn, Mo and Ag which are not found in the control plot are found in the fungicided plots, it is necessary to investigate hereafter how the heavy metallic components of fungicides are distributed in the plant food.

General Components

It seems from the results that the amount of total sugar and reducting sugar are affected by the dusting of fungicides in the plots of Cu-Hg, but, no distinct influence is found upon the other general components. And so, in

Table 17. Inorganic components in hulled rice.

Component \ Plot	Cu-Hg I	Cu-Hg II	Hg I	Hg II	Control
Ash*	1.5	1.5	1.5	1.6	1.5
Ca	>10	>10	>10	>10	>10
P	<10	$>$**10**	<10	<10	<10
Mg	$\leqq 10$	$\leqq 10$	$\leqq 10$	$\leqq 10$	$\leqq 10$
K	$\leqq 10$	$\leqq 10$	$\leqq 10$	$\leqq 10$	$\leqq 10$
Na	<1	<1	<1	<1	$\leqq 1$
Si	<1	<1	<1	$\leqq 1$	<1
Mn	<1	<1	<1	<1	<1
Pb	$<$ **0.01**	tr	$<$ **0.1**	$<$ **0.01**	–
Sn	–	–	tr	–	–
Al	<0.1	<0.1	<0.1	<0.1	<0.1
Zn	$<$ **0.1**	$<$ **0.1**	$<$ **0.1**	$\leqq$ **0.1**	<0.01
Mo	tr	tr	–	tr	–
Ni	–	–	–	tr	tr
Ti	tr	tr	<0.01	<0.01	<0.01
Sr	tr	tr	tr	tr	tr
Ag	$<$ **0.01**	tr	$<$ **0.01**	tr	–
Cu	$\leqq 0.01$	$\leqq 0.01$	$\leqq 0.01$	<0.01	<0.01
Fe	<0.01	<0.01	<0.01	$\leqq 0.01$	$\leqq 0.01$
Ba	–	–	–	–	–
Cr	–	–	–	–	–
Co	–	–	–	–	–
V	–	–	–	–	–
Hg	–	–	–	–	–

Note: 1) Figures show the gram per cent.

>10 : above 10 %
$\geqq 1$: 0.1 ~1.0 %
<0.1 : 0.01 ~0.1 %
<0.01 : 0.001~0.01%
tr : trace
– : unable to be found out by this method.

2) Figures which differ remarkably compared with those of the control are shown in gothic types.

3) The values of this table are rough values because the conditions of the quantitative radiation of the elements differ from each other at the time of spectral analysis.

* Is shown by gram per cent of dry hulled rice.

Table 18. General components in hulled rice.

Plot \ Component	Moisture	Protein*	Fat*	Saccharide*			Ash*
				Total-sugar	Reducing-sugar	Nonreducing-sugar	
Cu-Hg I	13.0	9.5	2.6	88.5	0.82	2.31	1.5
Cu-Hg II	13.2	9.0	2.9	87.5	0.82	1.58	1.5
Hg I	13.6	9.3	3.1	102.0	0.72	1.77	1.5
Hg II	12.9	9.1	3.5	91.5	0.60	1.88	1.6
Control	12.5	8.7	2.9	91.2	0.75	2.49	1.5

* Is shown by gram per cent of dry hulled rice.

general, it is conjectured that the dusting of fungicides does not affect the general components of hulled rice.

Vitamins

From Table 19, it is found that the fungicided plots give values a little higher than the control concerning the amounts of thiamine, but, the amounts of riboflavin give the opposite tendency. As to the kinds of fungicides, the

Table 19. Vitamin in hulled rice.

Plot / Component	Cu-Hg I	Cu-Hg II	Hg I	Hg II	Control
Thiamine	270	264	244	279	237
Riboflavin	45	52	69	57	83

Note: Values are shown by μg per 100 g of dry hulled rice.

Hg plot gives the higher value of the quantity of the riboflavin than the Cu-Hg plot and it is permissible to infer that the amount of riboflavin in hulled rice is affected by the dusting of fungicides. This influence of fungicides upon the content of riboflavin in hulled rice is by far most remarkable among those of the factors described above.

Katagiri (22) previously reported that the existence of Fe^{++} prevents the biosynthesis of riboflavin of the microorganism and on the contrary Rokusyo and others (23) found that the addition of Pb^{++} promotes the biosynthesis of riboflavin of the same microorganism, and they inferred that a delicate quantitative relation exists between the amount of biosynthesis of riboflavin by microorganism and the amount of Fe^{++}, Pb^{++} and the other inorganic components dissolved in water. The same fact is also expected of rice plants. In this experiment, it is found that the content of riboflavin in hulled rice is affected by the dusting of fungicides, but, because the Hg could not be estimated, it was not certified whether the biosynthesis and accumulation of riboflavin in rice plants are affected by the existence of Hg, which is the main component of fungicides, or affected by the existence of Pb, Sn, Mo and Ag, which is conjectured to be absorbed from the impurities of fungicides. About these questions, further investigations are necessary.

3. Summary

Varieties of rice plants and other environmental factors influence more or less upon the content of riboflavin in hulled rice and it was found that the more remarkable difference of the influence of the above factors appears in the case of the culture in peat soil than in clayey loam. Namely, the amount of riboflavin in hulled rice differs according to the difference of adaptability to

the growth environment of every variety, but in general, in the case of clayey loam it seemed that the difference of variety affects comparatively stronger than the differences of geographical location and year. Especially, the specific characters of the variety was markedly observed in the peat soil. The influence of the fertilizers upon the content of riboflavin in hulled rice is less than that of the variety, but, fungicides give nearly the same degrees of influence as that of the variety and thus it is conjectured that the content of riboflavin in hulled rice is mainly affected by the two factors, the variety and a fungicide.

Acknowledgements

I wish to thank Prof. Hisashi Ariyama for his cordial guidance during the present study, to Prof. Akio Fujiwara and Assistant Prof. Shin Nagasawa for their pertinent advices, and also Prof. Nobuhisa Maeda, Instructors Toshio Sugawara, Tae Goto and Messrs. Yasuo Takijima, Kunio Negishi, Shigeyoshi Ikeda, Akisuke Araki, Miss. Hisako Takahashi and Mr. Syuichi Kimura for their cooperation.

References

1) Crane, M.B. and Zilva, S.S. (1931). J. Pom. and Hort. Sci., **9**.
2) Mangelsdorf, P.C. and Fraps, G.S. (1931). Sci., **73**.
3) Le Rosen. (1941). Proc. Nat. Acad. Sci., **27**.
4) Walker, J.C. and R.E. Foster. (1946). Am. J. Bot., **33**, 758.
5) Sugawara, T. (1957). Studies on the Vitamin C in Field and Garden Crops, pp. 26—37, Yokendo Co. Ltd., Tokyo. (in Japanese)
6) Wade, B.L. *et al.* (1951). Southern Cooperative Series Bulletin, No. 15; (1953). C.A., **48**, 300—h.
7) Gastler, G.F. and A.W. Helverson (1952). Proc. S. Dakota Acad. Sci., **31**, 182; (1954). C.A., **48**, 3580—e.
8) Krober, O.L (1956). J. Agr. Food Chem., **4**, 254.
9) Tandon, O.B. *et al.* (1957). ibid., **5**, 137.
10) Adams, D.F. *et al.* (1957). ibid., **5**, 108.
11) Peterson, W.J. *et al.* (1948). Am. Fertilizer. **109**, 24; (1950). Bio. Abst., **24**, 7315.
12) Tanitazawa, M. and S. Tono (1952). J. Sci. Soil and Man, **22**, 191.
13) Sone. K. (1958). Tohoku J. Agr. Res., **9**, 93.
14) Craft, A.S. (1953). J. Agr. Food Chem., **1**, 51.
15) Richard J. Aldrich (1953). ibid., **1**, 257.
16) Ginsburg, J.M. (1955). ibid., **3**, 322.
17) Elsie H. Dawson *et al.* (1953). ibid., **1**, 399.
18) Haward Reynolds, G.L. Gilpin and Irwin Hornstein (1953). ibid., **1**, 772.
19) Kirkpatrick, M.E. *et al.* (1955). ibid., **3**, 409.
20) Gilpin, G.L. *et al.* (1957). ibid., **5**, 44.
21) Birdsall, J.J. *et al.* (1957). ibid., **5**, 523.
22) Katagiri, E. and K. Imai (1952). Vitamins, **5**, 224. (in Japanese)
23) Rokusyo, B. *et al.* (1952). J. Ferm. Tech., **30**, 94. (in Japanese)
24) Okamura, T. (1940). Ohara Nogyo Kenkyuzyo Tokubetsu Hokoku, No. 5, pp. 517. (in Japanese)
25) Ferri, M.G. *et al.* (1950). An. Acad. Brasil Cienc., **22**, 161; (1951).

Bio. Abst., **25**, 9133.
26) Galston, A.W. (1949). Am. J. Bot., **36**, 824 ; (1950). Bio. Abst., **24**, 13460.
27) Sone, K. (1956). J. Jap. Soc. Food Nutr., **8**, 270. (in Japanese)
28) (ed.) Laboratory of Agricultural Chemistry, Faculty of Agriculture, the University of Tokyo (1949). Nogei Kagaku Bunseki Syo, Asakura Syoten Co. Ltd., Tokyo. (in Japanese)
29) Sakurai, Y. *et al.* (1941). J. Sci. Res. Inst., **20**, 308.
30) Goto, T. (1954). J. Jap. Soc. Food Nutr., **7**, 69.